# Understanding the Immunological Basis of the Medical Revolution Occurring in Cancer Immunotherapy:

## Implications for Ultrasound, Radiation and Other Biophysical Therapies.

Elizabeth A. Repasky, Ph.D.
William Huebsch Professor of Immunology
Program Leader: Cell Stress and Biophysical Therapies
elizabeth.repasky@roswellpark.org

## Current strategies to combat cancers

**Mechanics -- surgery, 1600BC**
**Physics -- radiotherapy, 1896**
**Chemistry -- chemotherapy, 1942**
**Biology -- immunotherapy, ~ mid-1970s**

Immunotherapy is currently undergoing a period of remarkable therapeutic success. The resultant "medical revolution" has implications for how- *and if*- all other therapies, including biophysical therapies, will be applied in the future.

## Tumor Antigens Exist and are Immunogenic

**Tumor-specific: TSA**

Oncogenic mutants of normal cellular genes:ras, bcr-abl, p53
Randomly mutated genes: TSTA´s (tumor-specific transplantation antigens)

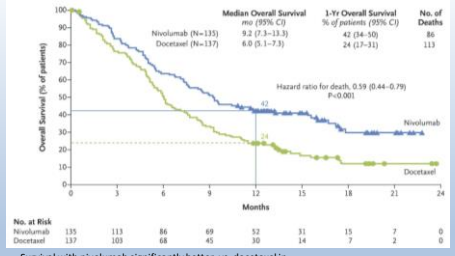
Can be identified: biochemical
cDNA cloning

**Tumor-associated: TAA**
Normal cellular proteins aberrantly expressed

Tyrosinase - melanomas (enzyme melanin biosynthesis)
Cancer/testis antigens: expressed testis and trophopblasts
Oncofetal antigens: developing fetal tissue
CEA: carcinoembryonic antigen - colo and many cancers,
AFP: α-fetoptotein - hepatocellular cancer and others
not specific, can be induced inflammatory conditions
Altered glycolipid and glycoprotein antigens:
gangliosides - in melanomas
Mucin-1 - O-linked carbohydrates
Tissue-specific differentiation antigens

## Immunotherapy (& Immunology) at the Center Stage of Cancer Therapy

- FDA approvals: Provenge, CTLA4 blockade, PD1/PDL1 blockers
- Big Pharma & Biotech Enter Cell-based Immunotherapies (DC, CAR-T, TIL...)
- 2013 Science Breakthrough of the Year; Time Magazine Cover Story- April 4th, 2016
- 2011 Nobel Prize: Ralph Steinman (Dendritic cell function)
- 2015 Lasker Award- James Allison

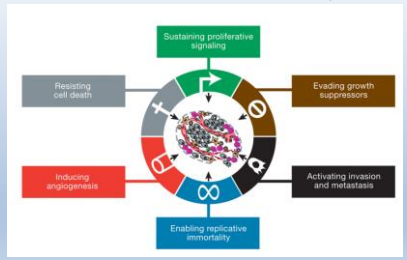




Survival with nivolumab significantly better vs. docetaxel in patients with previously treated squamous-cell NSCLC

P < 0.001

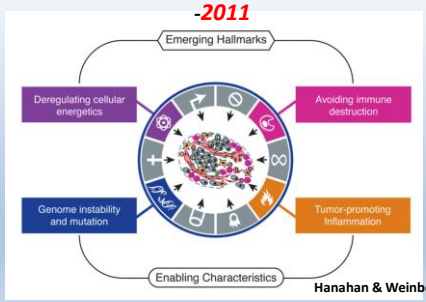
Brahmer et al, NEJM 2015

**Original 6 Hallmarks of Cancer—no mention of a role for evasion of immunity!**



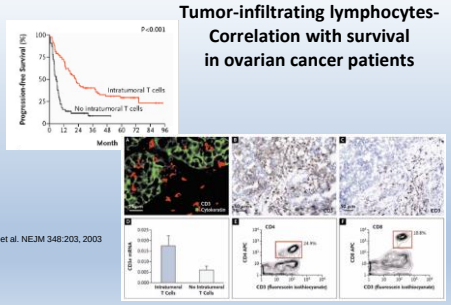

Hanahan & Weinberg Cell 2000

**Hallmarks of Cancer- *The Next Generation -2011***




**Hanahan & Weinberg Cell 2011**

**Tumor-infiltrating lymphocytes- Correlation with survival in ovarian cancer patients**

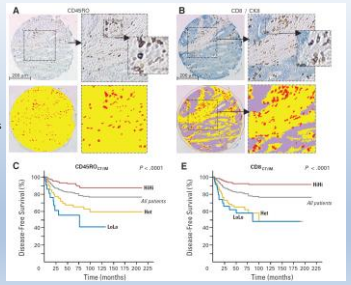



Zhang et al. NEJM 348:203, 2003

## T-cell infiltrate correlates with 5 yr outcome

IHC

Image analysis



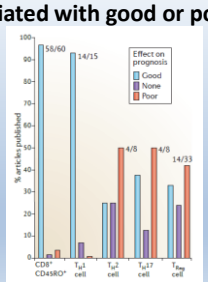

Pages…/Galon J Clin Oncol. 2009

## Cancer classification using the "Immunoscore": a worldwide task force

- Currently histopathological stage scoring is based on TNM (Tumor, Node, Metastasis)
- Patients of same stage can have very different outcomes
- Little value in predicting response to therapy
- Long-term outcome involves immune response
- "Immunoscore"= immunological biomarker

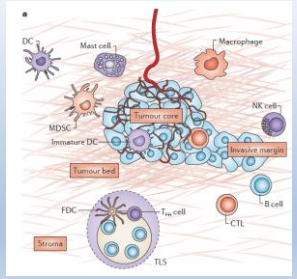
Galon et al. J Trans Med, 2012

## Different immune cell infiltrates are associated with good or poor prognosis




Fridman/Galon Nat Rev Cancer 12 (2012)

## The Immune Contexture




Fridman/Galon Nat Rev Cancer 12 (2012)

| Immune contexture | Parameters: positive association with survival |
|---|---|
| Type | CTLs ($CD3^+CD8^+$) |
| | Memory T cells ($CD45RO^+$) |
| Location | Core of the tumour |
| | Invasive margin |
| Density | Number of cells per $mm^2$: 1, 10, 100, 1,000, 10,000; $CD3^+_{CT}$, $CD3^+_{IM}$, $CD8^+_{CT}$, $CD8^+_{IM}$, $CD45RO^+_{CT}$, $CD45RO^+_{IM}$ |
| Functional orientation | $T_H1$ cell-associated factors (IFNγ, IL-12, T-bet and IRF1) |
| | Cytotoxic factors (granzymes, perforin and granulysin) |
| | Chemokines (CX3CL1, CXCL9, CXCL10, CCL5 and CCL2) |
| | $T_H17$ cells, $T_{reg}$ cells and $T_H2$ cells have a variable effect on survival, depending on tumour type |
| TLS | Presence and quality |

Fridman/Galon Nat Rev Cancer 12 (2012)

## Immunotherapy: Many approaches currently in progress

- Checkpoint Inhibitors ✓
- Oncolytic Viruses ✓
- Bi-specific Antibodies ✓
- Cancer Vaccines ✓
- CAR-T
- Natural Killer Cell
- T-cell Receptors
- DART
- STING
- Cytotoxic T-cells
- Tumor Infiltrating Lymphocytes
- Cytokines
- More…..

✓ = FDA Approved

**<u>The challenges:</u> Even though immunotherapy is resulting in remarkable outcomes in deadly cancers, only a subset of patients respond. And, some cancers appear more resistant than others. Also, the toxicity (immune adverse effects) can be significant in many patients.**

Some basic tumor immunology leading to the current immunotherapies;

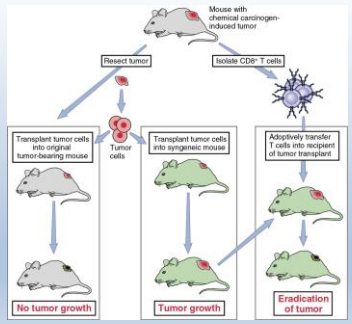
Can Radiation, Chemotherapy, HIFU, RF Ablation be used to improve outcome of Immunotherapy?

## Some Basic Concepts

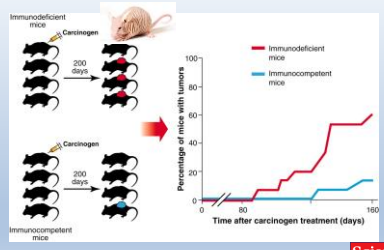
- The immune system can result in long lasting protection against many diseases.
- T Lymphocytes are particularly important for this long lasting memory against disease.
- Some pathogens, and cancer cells, have learned to escape the immune response.
- Cancer represents a dynamic equilibrium with the immune system. Influencing that balance can result in either long lasting cure, or progression of cancer.

A fundamental experiment which still drives the field of tumor immunology…..

For Background and Historical context: William Paul: A textbook of Immunology (Chapter on Tumor Immunology by Hans Schreiber, Univ. of Chicago)



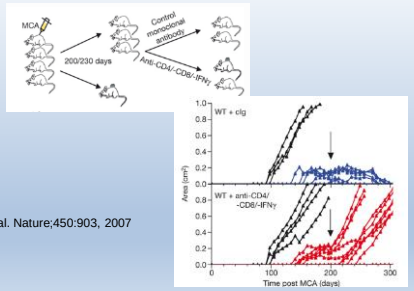
**The immune status of mice is a critical determinant of their susceptibility to tumors induced by chemical carcinogens.**




R D Schreiber et al. Science 2011;331:1565-1570

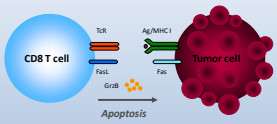
Published by AAAS

**T cells control latent tumors**

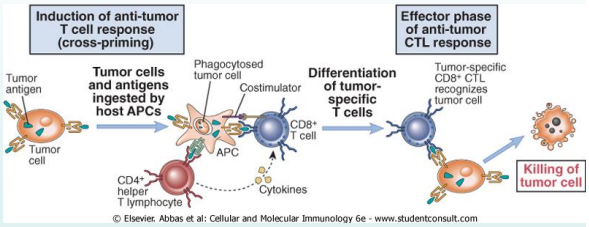



Koebel et al. Nature;450:903, 2007

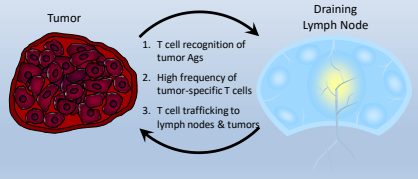
## Advantages of T Cell-Based Cancer Immunotherapy



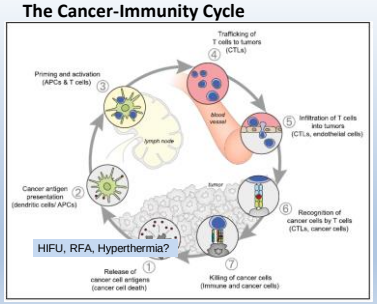

1. Exquisite specificity for target; limit collateral damage.
2. Target non-resectable tumors.
3. T cells can target tumors at sites throughout the body.
4. Long-lasting protection = Immunological Memory




## Adaptive Tumor Immunity:

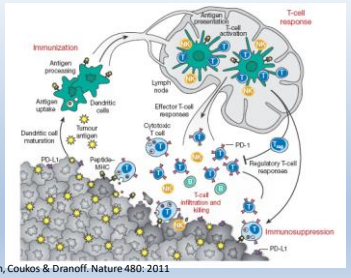



## The Cancer-Immunity Cycle



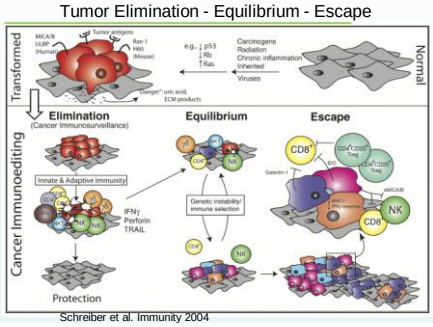

Chen and Mellman Immunity 39; 2013

## Tumor- Lymph node Interactions




Mellman, Coukos & Dranoff. Nature 480: 2011

## Tumor Elimination - Equilibrium - Escape

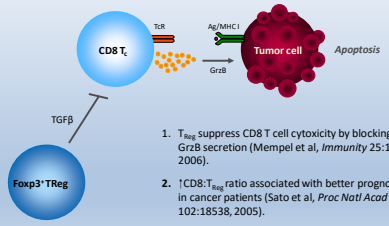



Schreiber et al. Immunity 2004

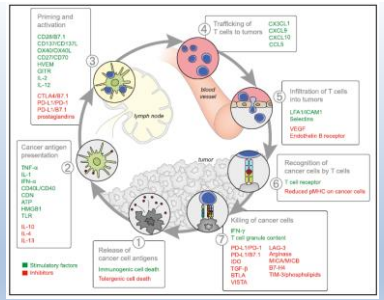
## Mechanisms of Tumor Escape from Immune Responses

- Loss of MHC or TAP
- Loss of co-stimulatory molecules
- Antigenic variation
- Secretion of immunosuppressive factors
  - e.g. TGF-b, IL-10
- T cells don't penetrate solid tumors
- Exhaustion of T cells
- T regulatory cells suppress anti-tumor responses

## Immunosuppressive $T_{Reg}$ Activity in the Tumor Microenvironment




1. $T_{Reg}$ suppress CD8 T cell cytoxicity by blocking GrzB secretion (Mempel et al, *Immunity* 25:129, 2006).
2. ↑CD8:$T_{Reg}$ ratio associated with better prognosis in cancer patients (Sato et al, *Proc Natl Acad Sci* 102:18538, 2005).

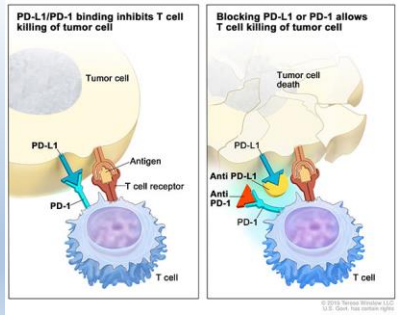


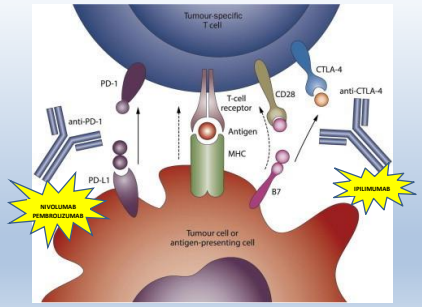

Chen and Mellman Immunity 39; 2013

## Immunotherapy: Checkpoint inhibitors

- Immune system relies on multiple checkpoints to avoid over activation on healthy cells
- Tumor cells hijack these checkpoints to escape detection
- CTLA-4 & PD-1 are upregulated on T cell surface in some cancers
- PD-L1 can be expressed on tumor cells endogenously or induced by association with T cells
- PD-1:PD-L1 interaction results in T cell suppression (anergy, exhaustion, death)

Atkins MB et al. Clinical Care Options slideset 2014.




Drake CG et al. Nat Rev Clin Onc. 2014;11:24-37.

## Approvals in 2016: the march of the checkpoint inhibitors

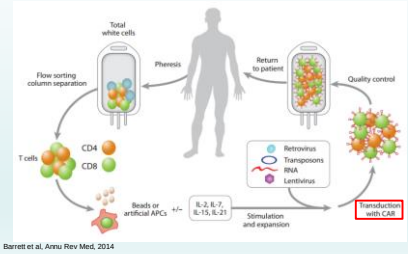
*Gideon M. Blumenthal and Richard Pazdur*

In 2016, FDA Oncology approved five new molecular entities and 17 efficacy supplements, including six accelerated approvals, 17 priority reviews, and 11 approvals of breakthrough-designated therapies. The FDA also approved five companion diagnostics, including a liquid biopsy test. One new anti-PD-L1 antibody was approved, along with six supplementary approvals of anti-PD-1/PD-L1 antibodies.

NATURE REVIEWS | CLINICAL ONCOLOGY
VOLUME 14 | MARCH 2017 | 131

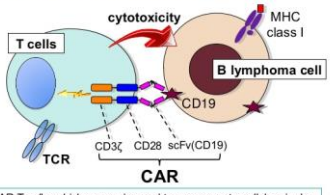
In the coming years, we are likely to see the continued expansion of the therapeutic landscape of immune-checkpoint inhibitors, targeted therapies, and novel companion and complementary diagnostics, including the further development of multiplex genomic testing platforms (including novel tissue-based or blood-based assays)

## CAR T cell transfer immunotherapy




Barrett et al, Annu Rev Med, 2014

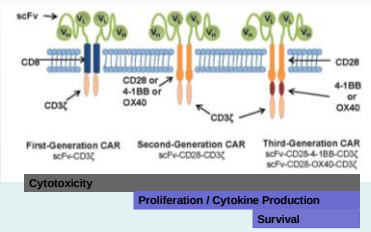
## Cytotoxicity of CD19-specific CAR-expressing T Lymphocytes against B Cell Lymphoma




CD19-CAR T cells, which are engineered to express extracellular single-chain immunoglobulin variable fragments to CD19, linked to cytoplasmic T cell activation domains including CD3-ζ, showed remarkable therapeutic benefits toward CD19+ B cell malignancies.

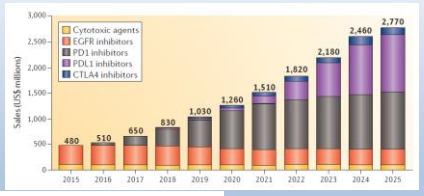
Ozawa K https://www.slideshare.net/spa718/4-keiya-ozawa

## 1st, 2nd, and 3rd generation CARs




Barrett et al, Annu Rev Med, 2014
Casucci et al, J Cancer, 2011
Park, Disc Med, 2010

The starting line for testing new therapies is changing fast...implications for new therapies?



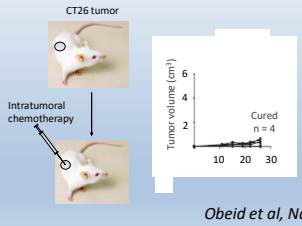

FROM THE ANALYST'S COUCH

The SCCHN drug market

*Jennifer Bamford and Rachel M. Webster*

April 2017

## The power of adaptive immunity in the response to chemotherapy

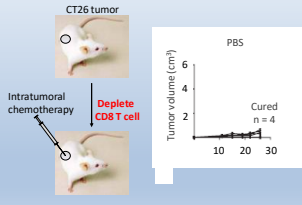






*Obeid et al, Nature Medicine, 2007*

## And, specifically the role of CD8+ T lymphocytes







*Obeid et al, Nature Medicine, 2007*

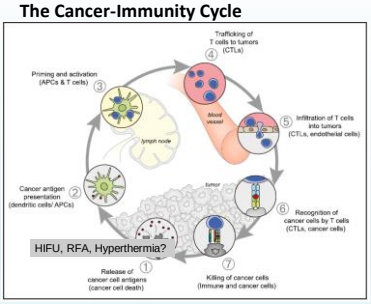
**Therapeutic effects of ablative radiation on local tumor require CD8⁺ T cells: changing strategies for cancer treatment**

Ralph R. Weichselbaum and colleagues: University of Chicago
2009 114: 589-595

blood

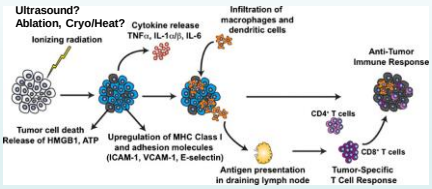
Developments in RT technology allow for the use of high-dose (or ablative) RT to target local tumors, with limited damage to the surrounding normal tissue. We report that reduction of tumor burden after ablative RT depends largely on T-cell responses. Ablative RT dramatically increases T-cell priming in draining lymphoid tissues, leading to reduction/eradication of the primary tumor or distant metastasis in a $CD8^+$ T cell–dependent fashion. We further demonstrate that ablative RT-initiated immune responses and tumor reduction are abrogated by conventional fractionated RT or adjuvant chemotherapy but greatly amplified by local immunotherapy.
Our study challenges the rationale for current RT/chemotherapy strategies and highlights the importance of immune activation in preventing tumor relapse. Our findings emphasize the need for new strategies that not only reduce tumor burden but also enhance the role of antitumor immunity.

**The Cancer-Immunity Cycle**



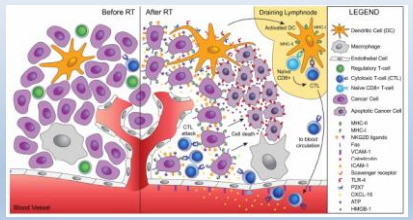

Chen and Mellman Immunity 39; 2013

**New paradigms concerning outcome from radiation therapy**




Adapted: Stephen L. Shiao & Lisa M. Coussens, J Mammary Gland Biol Neoplasia (2010) 15:411–421

**Ionizing radiation acts as a modifier of the tumor microenvironment converting the tumor into an *in situ* vaccine.**




Demaria & Formenti Frontiers in Oncology, 2012

## Irradiation and anti–PD-L1 treatment synergistically promote antitumor immunity in mice

JCI, 2014

Liufu Deng,[1] Hua Liang,[1] Byron Burnette,[1] Michael Beckett,[1] Thomas Darga,[2] Ralph R. Weichselbaum,[1] and Yang-Xin Fu[2]

[1]Department of Radiation and Cellular Oncology, The Ludwig Center for Metastasis Research, and [2]Department of Pathology, University of Chicago, Chicago, Illinois, USA.

Am J Clin Oncol. 2015 Feb;38(1):90-7. doi: 10.1097/COC.0b013e3182868ec8.

**Immune-priming of the Tumor Microenvironment by Radiotherapy: Rationale for Combination With Immunotherapy to Improve Anticancer Efficacy.**

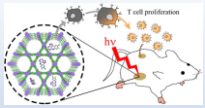
Shahabi V[1], Postow MA, Tuck D, Wolchok JD.

International Journal of Radiation Oncology biology • physics

www.redjournal.org

**Anti-PD-1 Blockade and Stereotactic Radiation Produce Long-Term Survival in Mice With Intracranial Gliomas**

Zeng et al, 2013



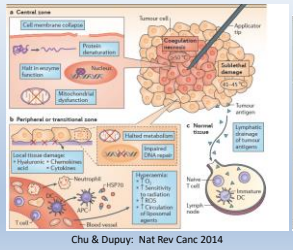

**Chlorin-Based Nanoscale Metal–Organic Framework Systemically Rejects Colorectal Cancers via Synergistic Photodynamic Therapy and Checkpoint Blockade Immunotherapy**

Kuangda Lu[†§], Chunbai He[†§], Nining Guo[†§], Christina Chan[†], Kaiyuan Ni[†], Ralph R. Weichselbaum[‡], and Wenbin Lin[*†]

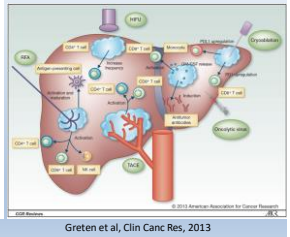
[†] *J. Am. Chem. Soc.*, 2016, 138 (38), pp 12502–12510

Here we describe a treatment strategy that combines PDT by a new chlorin-based nanoscale metal–organic framework (nMOF), TBC-Hf, and a small-molecule immunotherapy agent that inhibits indoleamine 2,3-dioxygenase (IDO), encapsulated in the nMOF channels to induce systemic antitumor immunity. The synergistic combination therapy achieved effective local and distant tumor rejection in colorectal cancer models. We detected increased T cell infiltration in the tumor microenvironment after activation of the immune system with the combination of IDO inhibition by the small-molecule immunotherapy agent and immunogenic cell death induced by PDT. We believe that nMOF-enabled PDT has the potential to significantly enhance checkpoint blockade cancer immunotherapy, affording clinical benefits for the treatment of many difficult-to-treat cancers.

## Potential of ablative therapies to activate immune cells

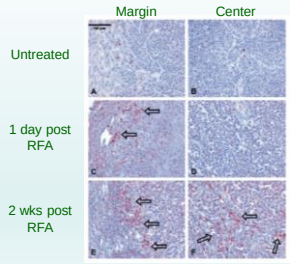


Chu & Dupuy: Nat Rev Canc 2014



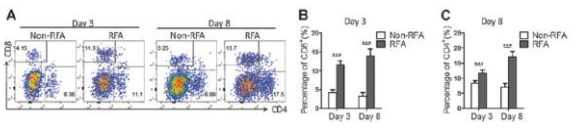
Greten et al, Clin Canc Res, 2013

## RF Ablation Increases T Cell Infiltration Into Tumor Tissues



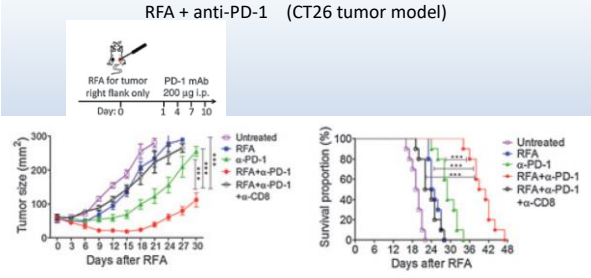

*Wissniowski et al., Cancer Research, 2003; 63: 6496-500*

## RF Ablation alters immune infiltrate



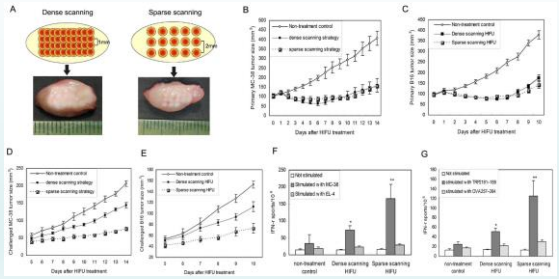

Shi et al, 2016 Clin Canc Res

RFA + anti-PD-1 (CT26 tumor model)



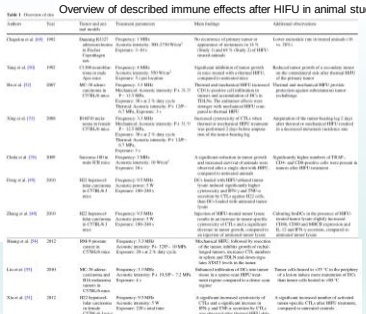

Shi et al, 2016 Clin Canc Res

HIFU scan strategies impact systemic immune response




Liu et al, J Trans Med 2010

Overview of described immune effects after HIFU in animal studies



From van dem Bokgaart et al, 2017,
Cancer Immunol.Immunother.

Overview of described immune effects after HIFU in clinical studies

| Authors | Year | Patient information | HIFU parameters | Main findings | Additional observations |
|---|---|---|---|---|---|
| Rosberger et al. [59] | 1994 | 5 patients with choroidal melanoma | Exposure: >50 °C for 5 min | $CD4^+/CD8^+$ ratio reverted to normal after HIFU in 2 of 5 patients with an abnormal $CD4^+/CD8^+$ ratio | |
| Wang et al. [61] | 2002 | 15 patients with late-stage pancreatic carcinoma | Frequency: 1.1 MHz<br>Acoustic power: 500–1600 W<br>Exposure: 30–80 s per location | A significant increase in the activity of NK cells after HIFU treatment | Nonsignificant increase in $CD3^+$ and $CD4^+$ T cells in 66 % of patients (10/15) |
| Wu et al. [71] | 2003 | 23 female patients with biopsy-proven breast cancer | Frequency: 1.6 MHz<br>Acoustic intensity: 5000–15,000 W/cm$^2$<br>Exposure: 30–180 min total time | HIFU-treated tumors showed significant decrease in PCNA, CD44v6, MMP-9 and erbB2 mRNA levels | |
| Kramer et al. [45] | 2004 | 6 patients with prostate cancer | Frequency: 4 MHz<br>Acoustic intensity: 1260–2000 W/cm$^2$<br>Exposure: 4 s per location | A significant upregulation of HSP-22 and -73 at the border zone of HIFU-induced thermal lesion in prostate cancer patients | |
| Wu et al. [60] | 2004 | 16 patients with solid malignancies | Frequency: 0.8 MHz<br>Acoustic intensity: 5000–20,000 W/cm$^2$<br>Exposure: 2.5–8 h total time | A significant increase in $CD4^+$ T cells after HIFU treatment | $CD4^+/CD8^+$ ratio reverted to normal after HIFU in 3 patients with an abnormal $CD4^+/CD8^+$ ratio |
| Zhou et al. [66] | 2008 | 15 patients with various solid malignancies | Frequency: 0.8–1.2 MHz<br>Acoustic intensity: 140–260 W<br>Exposure: 4–39 min total time | A significant decrease in serum VEGF, TGF-$\beta$1 and -$\beta$2 cytokine levels after HIFU treatment | |
| Wu et al. [31] | 2007 | 23 female patients with biopsy-proven breast cancer | Frequency: 1.6 MHz<br>Acoustic intensity: 5000–15,000 W/cm$^2$<br>Exposure: 45–150 min total time | HSP-70 expression was detected on the ablated cancer cells in all patients treated with HIFU | No expression of CD44v6, MMP-9 and PCNA in HIFU-treated tumors |
| Lu et al. [62] | 2009 | 23 female patients with biopsy-proven breast cancer | Frequency: 1.6 MHz<br>Acoustic intensity: 5000–15,000 W/cm$^2$<br>Exposure: 45–150 min total time | A significant increase in $CD3^+$, $CD4^+$ and $CD8^+$ T lymphocyte infiltration in the tumor, compared to controls | Increased numbers of NK cells and FasL+, granzyme+, perforin+ TILs found in HIFU-treated tumors |
| Xu et al. [63] | 2009 | 23 female patients with biopsy-proven breast cancer | Frequency: 1.6 MHz<br>Acoustic intensity: 5000–15,000 W/cm$^2$<br>Exposure: 45–150 min total time | A significant increase in infiltration and activation of macrophages and DCs in HIFU-treated tumors, compared to controls | |
| Wang et al. [64] | 2013 | 120 patients with uterine fibroids | Frequency: 0.8 MHz<br>Maximum acoustic power: 400 W<br>Exposure: not stated | Serum levels of IL-6 and -10 increased after HIFU treatment | IL-2 serum levels remained stable in HIFU-treated patients, compared to the patients receiving surgical resection where the IL-2 levels decreased |

From van den Bokgaart et al, 2017, Cancer Immunol Immunother

**The Importance of Dosimetry Standardization in Radiobiology**

Marc Desrosiers, Larry DeWerd, James Deye, Patricia Lindsay, Mark K. Murphy, Michael Mitch, Francesca Macchiarini, Strahinja Stojadinovic, and Helen Stone. Journal of Research of the National Institute of Standards and Technology, 2013

1) Radiation equipment and methods are increasing in variety and complexity.
2) Radiation biologists rarely receive training in radiation dosimetry.
3) Radiation biologists usually use irradiation equipment dedicated to research that is not shared with and calibrated by their clinical colleagues.
4) Radiobiologists now rarely work with radiation physicists as part of their joint routine duties, and there are fewer radiation physicists who are trained in the unique characteristics of the equipment used and problems involved in performing dosimetry in support of radiation biology.

***As with the collaboration between the biologist and statistician, which aids in determining the required sample size of the experiments, <u>the biologist-physicist collaboration can aid in determining the accuracy and precision required by a given experimental design and the methods needed to achieve these</u>.***

A growing awareness of problems in reproducibility of pre-clinical research, including cancer research

AAPM 2017 JUL 30–AUG 3

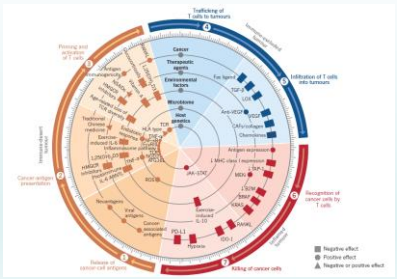
CONNECTING OUR PATHWAYS. UNIFYING OUR PROFESSION.

59TH ANNUAL MEETING & EXHIBITION | DENVER, CO

editors. Requiring better reporting of animal studies will raise awareness of the importance of rigorous study design to accelerate scientific progress."

**The "cancer-immune set point"**

Chen & Mellman, Nature 2017



**Thank you!**

**QUESTIONS?**